Claims were rejected under 35 USC 112, 1st ¶, for allegedly lacking enablement. Reconsideration is requested.

Satisfaction of enablement under 35 U.S.C. 112, first paragraph,

> requires nothing more than objective enablement. . . . [A] specification . . . *must* be taken as complying with . . . 35 U.S.C. 112 *unless* there is reason to do doubt the objective truth of statements relied upon therein.

Staehelin v. Secher, 24 USPQ2d 1513, 1516 (BPA & I 1992) (*emphasis in original*). In order to sustain a rejection for lack of enablement under §112, first paragraph, the PTO must cite evidence in support of any allegations of non-enablement, in addition to explaining *why* it doubts the truth of statements of enablement made in the specification. *In re Sichert*, 196 USPQ 209 (CCPA 1977).

Lack of enablement is not demonstrated merely because the claim scope might, theoretically, cover embodiments that do not work; the function of the claims is not to specifically exclude possibly inoperative embodiments. *Atlas Powder v. E.I. du Pont de Nemours Co.*, 224 USPQ 409 (Fed. Cir. 1984). Even in an unpredictable area, such as chemistry, the PTO must advance reasons why a patent applicant's broad assertion of enablement is not true. *In re Bowen*, 181 USPQ 48 (CCPA 1974). In order to sustain a rejection for lack of enablement under §112, and shift the burden to a patent applicant, the PTO must advance evidence or reasoning inconsistent with enablement. *Sichert, supra.*

Lack of enablement under §112 is not established by mere allegations of undue breadth, that is, by merely arguing that claims read on non-disclosed embodiments. *Horton v. Stevens*, 7 USPQ2d 1245 (BPA & I 1988). In order to satisfy the requirements of §112, first paragraph, "it is not necessary to embrace in the claims or describe in the specification all possible forms in which the claimed principle may be reduced to practice." *Smith v. Snow*, 294 U.S. 1, 11 (1935). The law does

not require an applicant to describe in his specification every conceivable embodiment of the invention. *SRI Int'l v. Matsushita Elec. Corp. of America*, 227 USPQ 577, 586 (Fed. Cir. 1985).

Enablement under § 112 of the statute is determined from the viewpoint of one of ordinary skill in the art at the time of filing the application for patent, i.e., at the time of constructive reduction to practice. The person of ordinary skill in the art brings with him a knowledge and understanding of the entirety of the prior art up until the date of application.

Since the skilled artisan is well aware of what is already known in the art, providing the same information in a patent specification would be redundant. Thus, while working examples drawn to specific embodiments may be desirable, they are not *required* in order to satisfy enablement under §112. *In re Strahilevitz*, 212 USPQ 561 (CCPA 1982). It is well established that working examples are not necessary when one possessed of knowledge of ordinary skill in the art could practice the invention without the exercise of undue experimentation. *Ex parte Nardi*, 229 USPQ 79 (BPA & I 1986). "In satisfying the enablement requirement, an application need not teach, and preferably omits, that which is well known in the art." *Staehelin*, 24 USPQ2d at 1516. In "satisfying the enablement requirement, an application need not teach, and preferably omits, that which is well known in the art." *Staehelin*, 24 USPQ2d at 1516. A "patent need not disclose, and preferably omits, that which is well known in the art." *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81, 94 (Fed. Cir. 1986). Moreover: "Non-critical features of the invention may be supported by a more general disclosure than those at the heart of the invention." *In re Stephens*, 188 USPQ 659, 661 (CCPA 1976).

Applicants, further, respectfully direct the examiner's attention to the PTO guidelines concerning rejections under §112, 1st ¶, for lack of enablement for "using" the invention, which is

the basis of the instant rejections under §112, ¶1. These guidelines apply when the rejection concerns the *use* of the invention, regardless of whether the rejection is under §112 or §101 of the statute. This is made clear in the "Guidelines," themselves; MPEP 706.03(a)(1) is entitled:

> Guidelines For Examination of Applications For Compliance With the Utility Requirements of 35 U.S.C. 101 ***and 35 U.S.C. 112***

Keeping the foregoing in mind, specifically, the guidelines state (*emphasis added*):

> If the applicant has asserted that the claimed invention is useful for any particular purpose (i.e. a "specific utility") and that assertion would be considered credible by a person of ordinary skill in the art, do not impose a rejection based on lack of utility. Credibility is to be assessed from the perspective of one of ordinary skill in the art *in view of any evidence of record (e.g., data, statements, opinions, references, etc.)* that is relevant to the applicant's assertions.

In the present case, the statement of rejection alleges that the specification lacks guidance as to "how" the skilled artisan can practice the presently claimed invention, i.e., improve neural regeneration by inhibiting basal membrane formation induced by a lesion of the neural tissue. The rejection relies on Jackowski as the *evidence* of the alleged lack of enablement.

First of all, *any improvement* in neural regeneration achieved by the presently claimed invention is sufficient to satisfy enablement of "using" under §112, ¶1. "An invention need not be the best way or the only way to accomplish a certain result, and *it need only be useful to some extent* and in certain applications." *Carl Zeiss Stiftung v. Renishaw PLC*, 20 USPQ2d 1094, 1100 (Fed. Cir. 1991). Secondly, Jackowski teaches nothing inconsistent with the aforesaid utility of the presently claimed invention. Thus, the rejection under 35 USC 112, ¶1, cannot be sustained.

Claims were rejected under 35 USC 112, 2nd ¶, for allegedly containing indefinite claim language. Reconsideration is requested in view of the language used in the new claims presented,

hereby. Applicants submit that the language reflected in the new claims, taken together with the following remarks, resolves the issues raised in the statement of rejection.

According to the statement of rejection, claims are allegedly indefinite because they do not exclude certain subject matter. The claims cannot be rejected under §112, ¶2, for failing to exclude subject matter. The purpose of the claims is not to exclude possible inoperative subject matter. *Atlas Powder, supra*. *In re Smythe*, 178 USPQ 279, 286 (CCPA 1973). *In re Geerdes*, 180 USPQ 789, 793 (CCPA 1974).

Claims were rejected under 35 USC 102 for alleged anticipation based on each of Logan and Grumet. Reconsideration is requested.

For anticipation to exist each and every claim limitation, as arranged in the claim, must be found in a single prior art reference. *Jamesbury Corp. v. Litton Industrial Products, Inc.*, 225 USPQ 253 (Fed. Cir. 1985). The absence from a prior art reference of a single claim limitation negates anticipation. *Kolster Speedsteel A B v. Crucible Inc.*, 230 USPQ 81 (Fed. Cir. 1986). A reference that discloses "substantially the same invention" is not an anticipation. *Jamesbury Corp.* To anticipate the claim, each claim limitation must "*identically* appear" in the reference disclosure. *Gechter v. Davidson*, 43 USPQ2d 1030, 1032 (Fed. Cir. 1997) (*emphasis added*). While the skilled artisan may have sufficient knowledge to apply the teachings of an applicant's invention to the reference, "that presumed knowledge does not grant a license to read into the prior art reference teachings that are not there." *Motorola Inc. v. Interdigital Technology Corp.*, 43 USPQ2d 1481, 1490 (Fed. Cir. 1997). To be novelty defeating, a reference must put the public in possession of the identical invention claimed. *In re Donahue*, 226 USPQ 619 (Fed. Cir. 1985).

The statement of rejection alleges that the present invention as claimed would be anticipated by Logan et al; however, Logan et al discloses use of a *non-specific* agent, TGF-β. Therefore, Logan cannot anticipate the presently claimed invention since a method is claimed for the improvement of neuronal regeneration by "*specific* inhibition" (*emphasis added*) of basal membrane formation. This limitation being missing from the reference, there can be no anticipation.

According to the presently claimed invention, it was found, for the first time, that the basal membrane, which is induced by lesion of neuronal tissue, prevents regeneration of neuronal tissue. However, this is not even mentioned in the disclosure of Logan et al. Since Logan does not relate to the production of an extracellular matrix (ECM) in the CNS with the basal membrane, this reference cannot anticipate the present invention.

The specific neuronal regeneration, which is provided by the present invention, is also not possible with the teaching of Logan et al. Logan is silent with respect to the importance and relevance of the basal membrane. Also due to this fact, it cannot be anticipating the present invention.

With respect to Grumet et al., the reference reports about a neuron glia cell adhesion molecule. This molecule alone or in combination with one or more additional agents is useful in promoting the regeneration of a nerve in a subject having peripheral or spinal nerve damage. This cell-adhesion molecule binds to neuronal cells and molecules of the extracellular matrix (ECM).

However, contrary to the teaching of the presently claimed invention, Grumet does not deal with the reduction or construction of the basal membrane. Therefore, this reference also cannot anticipate the presently claimed invention, let alone that it does not provide the teaching to use a *specific* inhibitor of basal membrane formation to provide for regeneration of nerve cells. The

feature of the presently claimed invention that a specific interaction has to take place is very important, since a non-specific aggregation to constituents of the basal membrane occurs which may result in reinforcement and enlarging of the membrane (stopping of regeneration), but by no means in an induced reduction of the basal membrane.

Furthermore, Grumet teaches as essential a combination of NgCAM together with a nerve growth factor. This also is not the teaching of the presently claimed invention.

Claims were rejected under 35 USC 103 based on the teachings of Logan in view of White, Krause, and Kivirikko. Reconsideration is respectfully requested.

When conducting an obviousness analysis, "all limitations of a claim must be considered in determining the claimed subject matter as is referred to in 35 U.S.C. 103 and it is error to ignore specific limitations distinguishing over the [prior art] reference." *Ex parte Murphy*, 217 USPQ 479, 481 (PO Bd. App. 1982). In the context of a rejection for obviousness under §103, the "*Examiner* bears [both] the initial burden . . . of presenting a *prima facie* case of unpatentability" and "the ultimate burden of persuasion on the issue." *In re Oetiker*, 24 USPQ 1443, 1444 and 1447 (Fed. Cir. 1992), *emphasis, added*. "The Examiner can satisfy this burden only by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art *would lead* that individual to combine the relevant references. . . . Indeed, the teachings of the references can be combined only if there is some suggestion or incentive to do so." *Ex parte Obukowicz*, 27 USPQ 1063, 1065 (BPA&I 1992)(*emphasis, added*).

The "evidence upon which the examiner relies must clearly indicate that a worker of routine skill in this art would view the claimed invention as being obvious." *Ex parte Wolters*, 214 USPQ

735, 736 (BPA&I 1982). As explained by the Board in the decision *Ex parte Levengood*, 28 USPQ2d 1300, 1300-01 (BPA&I 1993)(*emphasis in original*):

> In order to establish a *prima facie* case of obviousness, it is necessary for the examiner to present *evidence*,[1] preferably in the form of some teaching, suggestion, incentive or inference in the applied prior art, that one having ordinary skill in the art *would have been led* to combine the relevant teachings of the applied references in the proposed manner to arrive at the claimed invention [*citations, omitted*].

An argument by the USPTO is "not prior art." *In re Rijckaert*, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993). When the

> USPTO asserts that there is an explicit or implicit teaching or suggestion in the prior art, it must indicate where such a teaching or suggestion appears *in the reference*. ... The mere fact that a certain thing *may* result from a given set of circumstances is not sufficient to establish inherency. ... [S]uch a retrospective view of inherency is not a substitute for some teaching or suggestion supporting an obviousness rejection.

28 USPQ2d at 1557, *emphasis added*. "It is facts which must support the legal conclusion of obviousness. *Ex parte Crissy*, 201 USPQ 689, 695 (POBdApp 1976)

> The Patent Office has the initial duty of supplying the factual basis for its rejection. It may not, because *it* may *doubt* that the invention is patentable, resort to speculation, unfounded assumptions or hindsight reconstruction to supply deficiencies in the factual basis.

In re Warner, 154 USPQ 173, 178 (CCPA 1967) (*emphasis in original*). When the claimed invention requires modification of the prior art, there is no obviousness under §103 when "[t]he prior art does not suggest . . . modification of the . . . [prior art], or provide any reason or motivation to make the modification." *In re Laskowski*, 10 USPQ2d 1397, 1398 (Fed. Cir. 1989). The fact that all elements of a claimed invention are known does not, by itself, make the combination obvious.

Ex parte Clapp, 227 USPQ 972 (BPA&I 1985). To support a rejection for obviousness based on the combination of separate prior art teachings, the USPTO "must identify specifically the principle, known to one of ordinary skill, that suggests the claimed combination." *In re Rouffet*, 47 USPQ2d 1453, 1459 (Fed. Cir. 1998). When "the examiner's comments regarding obviousness amount to an assertion that "one of ordinary skill in the relevant art would have been able to arrive at [the claimed] invention because he had the necessary skills to carry out the requisite process steps[,] [t]his is an inappropriate standard for obviousness." *Levengood*, 28 USPQ 2d at 1301.

> The mere fact that the prior art may be modified in the manner suggested by the Examiner does not make the modification obvious unless the prior art suggested the desirability of the modification.

In re Fritch, 23 USPQ2d 1780, 1783-84 (Fed. Cir. 1992).

Krause and White are not related to the field of the presently claimed invention. This reference teaches to avoid brain lesions by direct manipulation into the basic metabolism in order to avoid the destruction of cell-membrane. This shall be achieved by the administration of iron chelators (reactive oxygen species). Object of this teaching is the avoiding of cell damage, but not regeneration of nerves.

Since the skilled person did not have any incentive to combine the teachings of the different references, they cannot render obvious the presently claimed invention. The argumentation of the Examiner is expost factor analysis, in particular when he alleges that it would have been obvious to the person of ordinary skill in the art at the time the invention was made to modify the Logan et al method for using inhibitors such as taught in the other references. At first, there is no incentive for the skilled person to optimize the Logan reference. Furthermore, as the Examiner already points out, the prior art teaches the skilled person about inhibition of accumulation of *extracellular matrix*.

Thus, it becomes evident that the skilled person would not consider the basal membrane as taught by the presently claimed invention. Even if the skilled person would consider an optimization of the Logan process by combining the teachings of the other references, he or she would not come to the solution of the problem according to the presently claimed invention, which means that the basal membrane is the object to be considered.

White, Krause and Kivirikko do not construct any relation to the basal membrane which is the clue of the presently claimed invention.

The analysis given on page 14, last paragraph, of the Office action, i.e., that the person of ordinary skill would have been motivated to make the modification because iron-chelators would have allowed faster penetration of the blood brain barrier and may have caused fewer unwanted side effects compared to the other compounds, is so general that it cannot make obvious the presently claimed invention. Moreover, it does not have anything to do with the finding of the presently claimed invention with respect to the relevance of the reduction of the basal membrane regeneration of nerves, which have been damaged by lesions.

Favorable action is requested.

Respectfully submitted,

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A method for the improvement of neuronal regeneration

The present invention refers to a method for the improvement of neuronal regeneration, a medicament for the improvement of neuronal regeneration and use of a specific inhibitor substance.

Injury to adult mammalian CNS fiber tracts leads to the formation of a lesion scar consisting of a convoluted fringe of astroglial processes lined by a basal membrane (BM). This lesion scar is implicated as a major extrinsic constraint to effective axon regeneration in brain and spinal cord (1 - 4). While the dense astrocytic network is a permissive substrate for axon growth (5, 6), the presence of BM has been hypothesized as a crucial impediment for regeneration (7). However, experimental evidence was not shown. To the contrary, when the BM formed after a lesion of neuronal tissue was removed (24), no improved regeneration could be reproducibly monitored (25). Therefore, it is still of great importance to have a method for improving regeneration of injured neurons.

WO 93/19783 discloses a method for preventing, supressing or treating a CNS pathology characterized by a deleterious accumulation of extracellular matrix in a tissue by contacting the tissue with an agent that inhibits the extracellular matrix producing activity of TGF-ß. The disclosed methods can be used to prevent, suppress or treat scar formation in the CNS. As here useful agents are ~~addressed~~ neutralizing anti-TGF-ß antibodies, Arg-Gly-Asp-containing peptides, decorin and its functional equivalence such as biglycan and TGF-ß antagonists. TGF-ß has a wide spectrum of physiological functions such as activation of cell of the immune system, inhibition of cell proliferation, neurotrophic effects on sensory neurons, inhibition of Schwann cell myelination, anti-profilerative effects on glial cells,

immunsuppressive effects, stimulation of extracellular matrix deposition and chemoattraction of microglia cells. The anti-TGF-ß treatment would induce the opposite effects. Inhibition of TGF-ß activity leads to numerous non-specific cellular responses, which may even lead to unwanted side effects. One object of the invention is to avoid such potential unwanted side effects.

Surprisingly, improvement of regeneration of neuronal tissue after lesion is achieved by a method of the present invention.

According to the method of the invention improved regeneration of injured neuronal tissue is achieved by specific prevention or specific inhibition of basal membrane formation induced by a lesion of neuronal tissue.

The basal membrane is a structure which is composed of different elements. Elements of the basal membrane are collagen IV, laminin, entactin (Nidogen) accessory substances. The assembly of the elements to the basal membrane is performed by enzymes which may be assisted by cofactors.

Inhibitors of TGF-ß are not involved with a specific prevention or specific inhibition of basal membrane formation induced by lesion of neuronal tissue. According to the present invention it is achieved in an advantageous manner that a specific interaction is provided.

Preferably, the formation of the basal membrane is prevented or inhibited by applying a specific inhibitor substance of the synthesis of basal membrane building elements, or the assembly of basal membrane building elements, or both the synthesis of basal membrane building elements and the assembly of basal membrane building elements to a body in need thereof. The building elements of the basal membrane are in particular those which are involved with the formation of the basal membrane, for instance molecular structures building up the basal membra-

ne, such as monomeric compounds, accessory substances, substances for the assembly of the components of the basal membrane and the like.

In particular, the basal membrane building elements are selected from the group consisting of collagen IV, laminin, entactin, accessory substances for proper function, or the assembly of the basal membrane, or both the proper function and the assembly of the basal membrane.

A specific inhibitor substance of the invention is capable of preventing or inhibiting the formation of the basal membrane and/or is specifically interfering with the assembly process of the basal membrane. Preferably, the specific inhibitor substance is selected from the group consisting of antibodies against collagen IV, laminin, entactin, accessory substances for proper function, or the assembly of the basal membrane; Fe-chelating agents; inhibitors of amino acids hydroxylases, such as prolyl-4-hydroxylase, lysine-hydroxylase; 2-oxoglutarate competitors; antisense nucleotides or nucleotide analogs which are able to prevent or inhibit the expression of basal membrane building elements, and the like.

According to the invention there can further be used those inhibitor substances which are selected from the group consisting of N-oxaloglycine; Zn salts; pyridine derivatives, such as 5-arylcarbonyamino- or 5-arylcarbamoyl- derivatives, 2-carboxylate, 2,5 dicarboxylate, their ethyl esters or ethyl amides or -5-acyl sulfonamides, 2,4 dicarboxylate, their ethyl esters or ethylamides, or dimethoxyethylamides; 3,4 bipyridine, such as 5 amino-6-(1H)-one, 1,6-dihydro-2-methyl-6-oxo-5-carbonitril; 2,2'-bipyridine, such as 5,5'-dicarboxylic acid or its pharmaceutically acceptable salts, 4,4'-dicarboxylic acid ethyl ester or ethyl amide; 3,4'-dihydroxybenzoate, such as the diethyl ester; proline and its structural and functional analoges; ß-aminopropionitrile; desferrioxamine; anthracyclines; 2,7,8-trihydroxy anthraquinones, fibrostatin-C; coumalic acid or its pharmaceuti-

cally acceptable salts; 5-oxaproline, ß-lactam antibiotics.

In a preferred embodiment of the present invention the specific inhibitor substance(s) are applied in combination with one or more substances being capable of stimulating neuronal growth or inducing the expression of growth promoting proteins. Such neuronal growth stimulating substances are neurotrophic growth factors of the neurotrophin family and other growth factor families such as fibroblast growth factors, insulin and insulin-like growth factors, as well as epidermal growth factor, ciliary neuronotrophic growth factor (CNTF), glial cell-derived growth factor (GDNF), cytokines, neurotrophic proteoglycans and glycosamino-glycans, neural cell adhesion molecules like L1 (NILE), growth-associated proteins like GAP43 and anti-apoptotic proteins like bcl-2.

According to the invention it is preferred to locally apply the specific inhibitor substances in the neuronal tissue, intraventricularly, or systemically, in particular orally or intravenously.

The concentration of the specific inhibitor substance varies in view of the chemical nature. For example, antisense inhibitor substances may have more specific effects so that lesser amounts can be applied.

Typically, the specific inhibitor substance is applied in therapeutically effective amounts, such as 1 ng/kg to 1 mg/kg body weight, when low molecular compounds such as bipyridyl-derivatives are applied.

The invention also provides a medicament for the improvement of neuronal regeneration comprising a therapeutically effective amount of a specific inhibitor substance which is capable of prevention or inhibition of basal membrane formation induced by a lesion of neuronal tissue. Appropriate specific inhibitor substances are described hereinabove. The medicament may further

Large WGA-HRP-filled axon (arrowhead) in the mammillary body surrounded by compact myelin (arrows). d, e Electron micrographs of anterogradely WGA-HRP-labeled presynaptic terminals (arrowheads) in the mammillary body at 6 weeks after anti-Coll IV treatment. Scale bars, 100 μm (a), 50 μm (b), 0.1 μm (c), 0.5 μm (d), 1 μm (e).

Fig. 4: Electrophysiological properties of fornix fibers in unlesioned rats and lesioned/injected animals with regeneration. a, Schematic illustration showing the location of the stimulating (S) and recording (R) electrode at various conditions. b, Characteristic recordings of extracellular action potentials in a sagittal slice prepared from an animal with regeneration. Recordings were obtained under conditions as illustrated in a. Application of Tetrodotoxin (TTX) blocks the stimulus-evoked response. The net action potential is shown in trace 5. c and d, Distribution of conduction velocity and action potential response amplitude in unlesioned and lesioned/injected animals with regeneration.

The mechanically transected postcommissural fornix of the adult rat, a unidirectional and well-characterized fiber tract (8,9), was used to determine whether specific biochemical or immunochemical modulation of BM formation would provide a means to stimulate axon regeneration. Here we report that lesion-induced BM deposition can be significantly reduced by local injection of anti-collagen IV antibodies or α,α dipyridyl, an inhibitor of collagen triple helix formation and synthesis. Reducing the collagen network allowed massive axon elongation across the lesion site. The regenerating fornix fibers followed the original pathway, reinnervated their appropriate target, the mammillary body, were remyelinated and attained nearly normal conduction properties. On failure of adult mammalian CNS axons, we examined the spatio-temporal distribution pattern after penetrant CNS lesion and determined whether remodelling allows structural and functional regeneration of a transected CNS fiber tract.

Further preferred embodiments for restitution of functional circuitry after traumatic CNS lesion are the remyelination of regenerated fibers, the re-establishment of synaptic connections with the appropriate target and the restoration of normal conduction properties. Structural and functional properties of the regenerating axons were investigated using immunohistochemical, morphological and electrophysiological methods. Immunohistochemistry with an antibody against myelin basic protein demonstrated the remyelination of regenerated fornix axons along their entire length as early as 4 weeks after surgery (data not shown). This observation was confirmed by ultrastructural analysis of anterogradely WGA-HRP labeled axons in the distal stump which showed clear evidence of compact myelin sheath formation (Fig. 3c). In addition, ultrastructural studies provided evidence for the re-establishment of synaptic connections of regenerating axons within the mammillary body. Tracer reaction product was identified in presynaptic profiles with round vesicles that formed asymmetric synaptic junctions at unlabeled dendrites (Fig. 3d, e). The ultrastructural features of the labelled presynaptic profiles correspond to those described for the RA-type (round, asymmetric) of synaptic terminal, which is considered to be of subicular origin (8). The electrophysiological properties of regenerated fibers were studied using extracellular in vitro recording techniques applied to sagittal brain slices (400 μm) of 8 unlesioned rats and 4 treated animals showing regenerated fiber tracts. In unlesioned animals electrical stimulation of the fornix fibers elicited an extracellular action potential with an amplitude of 1.02 $\pm$ 0.14 mV and a conduction velocity of 0.48 $\pm$ 0.05 m/s (mean $\pm$ SEM, n=16, Fig. 4b-d). This axonal conduction velocity corresponds well to previously reported measurements (about 0.5 m/s for hippocampal Schaffer collaterals (15). Similar values for action potential amplitude and conduction velocity (1.12 $\pm$ 0.21 mV, 0.46 $\pm$ 0.1 m/s, n=5) were obtained in regenerating animals when the stimulating (S) and the recording (R) electrodes were positioned proximally to the lesion site (see S1 and R1 in Fig. 4a). In the latter animals, functionally intact fibers showing

axon

normal extracellular action potential amplitude and conduction velocity could also be demonstrated across (S3 and R3 in Fig. 4a; 0.8 ± 0.29 mV, 0.54 ± 0.14 m/s, n=3) and distal to the lesion site (S2 and R2 in Fig. 4a; 0.91 ± 0.24 mV, 0.43 ± 0.06 m/s, n=4) (Fig. 4c, d). In all animals, the stimulus-evoked extracellular responses were blocked by Tetrodotoxin, confirming their nature as Na+-dependent action potentials (Fig. 4b). From these data we conclude that the reorganization of the fornix tract is accompanied by structural and functional recovery of the regenerated axons.

Our results demonstrate that structural and functional restoration of lesioned mature fornix pathway can be achieved by reduction of BM formation in the lesion site. Data described here underscore the importance of extrinsic determinants in axonal regeneration but also demonstrates that once the axons have crossed the lesion scar other potential extrinsic regeneration constraints, like CNS myelin and oligodendrocytes (9,16-18), dense astrogliosis (6) and sulfated proteoglycans (19,20), do not impede their progress. The results further indicate that similar to other CNS circuits (21,22), fornix axons have an innate potential for regeneration and self-organization. These results give rise to new and promising concepts for therapeutic strategies that might contribute to the reduction of neurological deficits after CNS lesions.

The following examples are intended for further illustration of the invention but are not limiting.

Surgery. The left postcommissural fornix of 42 Wistar rats (180-210g) was transected stereotactically at a distance of about 1 mm proximal to the target, the mammillary body, using a Scouten wire knife as described previously (9). The completeness of transection was confirmed by serial reconstruction of the lesion site for each of the animals. Immediately after transection animals received a topical application (1.6 µl) of either polyclonal antibodies against collagen IV (anti-Coll IV, Bioge-

sections, deparaffinized and incubated as described above with a polyclonal anti-myelin basic protein (anti-MBP, Biogenex, 1:2) or anti-NF as primary antibodies. Specificity of the stainings was confirmed by omission of the primary antibody.

(boldface)

Electrophysiology and biocytin injections. Sagittal slices of 400 μm thickness were cut on a vibratome and maintained at 34-35°C in an interface-type recording chamber. Artificial cerebrospinal fluid (ACSF) consisted of (in mM) 124 NaCl, 3 KCl, 1.25 NaH_2PO_4, 1.8 $MgSO_4$, 1.6 $CaCl_2$, 26 $NaHCO_3$ and 10 glucose with a pH of 7.4 when saturated with 95% O_2 - 5% CO_2. Stimuli (100 μs, 5-20 V were delivered via a bipolar tungsten electrode. Extracellular action potentials were registered with a recording electrode (3-5 MW) located in the middle of the postcommissural fornix. Tetrodotoxin (TTX, Sigma) was applied locally in a concentration of 10 μM (dissolved in ACSF) with a broken micropipette placed on the slice surface near the recording site. Injections of a small biocytin (Sigma) crystal into the fornix were performed with a miniature needle. After an incubation period of 8-10 h in the interface chamber, slices were fixed in 4 % paraformaldehyde, resectioned and reacted with ABC peroxidase reagent (Vector Labs).

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